



## **Comparison of proficiency testing results on antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* obtained by laboratories from the ECDC FWD network (public health) and the EURL-AR network (veterinary/food) 2012**

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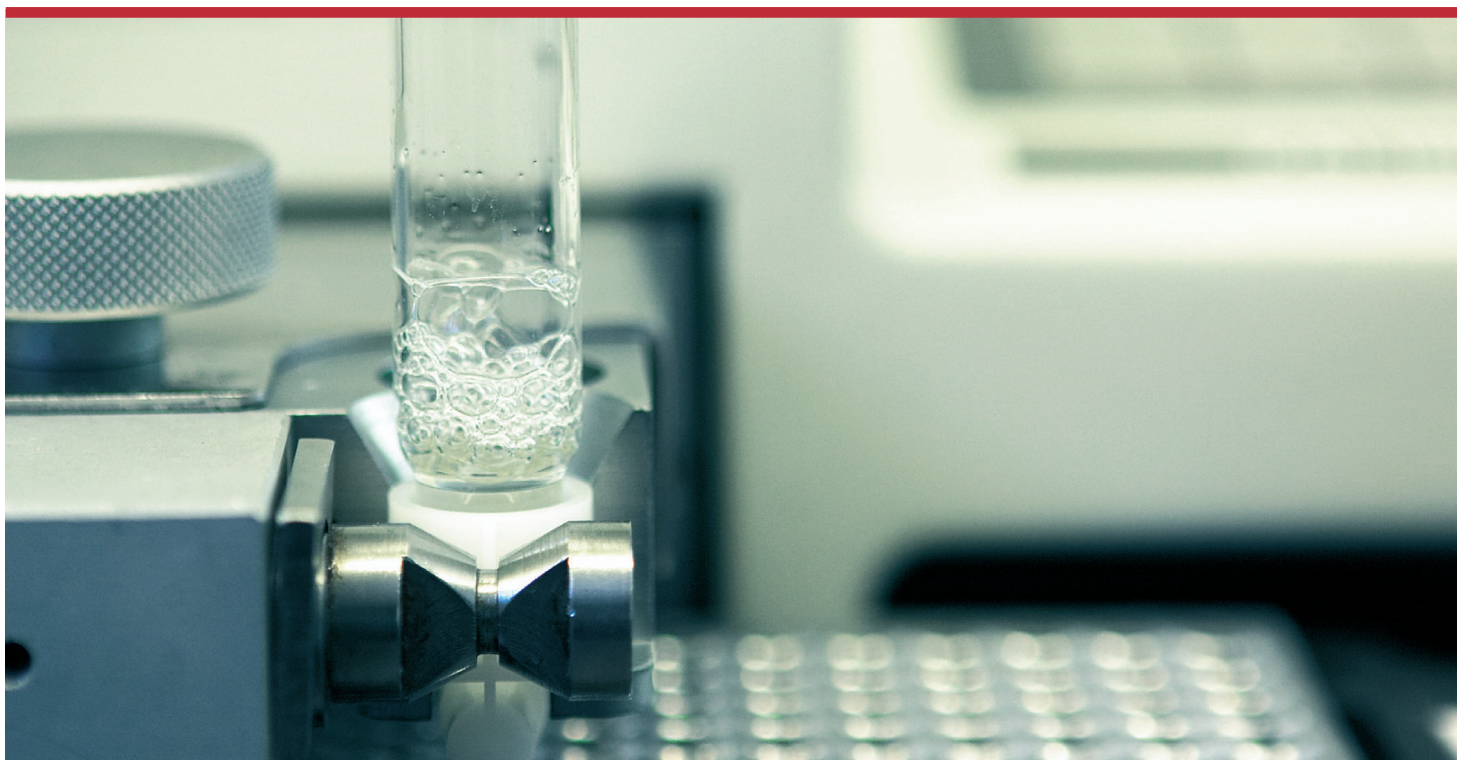
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COMPARISON OF PROFICIENCY TESTING RESULTS ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING OF *SALMONELLA* AND *CAMPYLOBACTER*  
OBTAINED BY LABORATORIES FROM THE ECDC FWD NETWORK  
(PUBLIC HEALTH) AND THE EURL-AR NETWORK (VETERINARY/FOOD) 2012



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## **European Union Reference Laboratory – Antimicrobial Resistance**

**Comparison of proficiency testing results on antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* obtained by laboratories from the ECDC FWD network (public health) and the EURL-AR network (veterinary/food) 2012**

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# 1. Introduction

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In this summary report, results are summarised and compared from the proficiency test trial conducted by the National Food Institute (DTU Food) as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR) aiming at two networks as participants, i.e. the *Salmonella* and *Campylobacter* laboratory contact points of the Food- and Waterborne Diseases and Zoonoses Network (FWD-network) under the coordination of European Centre for Disease Prevention and Control, and the EURL-AR network. The FWD-network consists of public health reference level laboratories, and the EURL-AR network consists of institutes from the veterinary/food sector.

Proficiency testing is considered an important tool for the production of reliable laboratory results of consistently good quality. This proficiency test focuses on *Salmonella* and *Campylobacter* and is the sixth External Quality Assurance System (EQAS) conducted for these microorganisms in the EURL-AR network. The public health laboratories were charged a fee to cover the expenses related to their participation in the *Salmonella* and *Campylobacter* antimicrobial susceptibility testing (AST) EQAS.

The objective of this EQAS was to assess and compare the quality of the antimicrobial susceptibility data produced by the reference laboratories and to identify areas which would require attention to produce reliable and harmonised susceptibility data.

At the annual EQAS conducted by the EURL-AR, the goal is to have each laboratory performing AST with less than 5% incorrect interpretations (interpretations deviating from

the expected results). This performance criterion has also been applied for the present report. Evaluation in detail of the obtained results from the EURL-AR network is presented in a separate report and is not the objective for the present report. This report will focus on the comparison of the obtained results between the two networks, i.e. between the public health sector and the veterinary-/food sector.

The data in this report are presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential. All conclusions are public.

Participants of an EQAS are expected to evaluate their own results and introduce corrective actions if necessary. The categorization of an uploaded interpretation as incorrect in the EURL-AR EQAS should induce the participant to perform a self-evaluation. This self-evaluation could very well include a comment on the fact that an acceptable deviation for MIC-determination is  $\pm$  one dilution step, which in some cases may affect the interpretation of the result. Therefore, the self-evaluation may lead to arguments which can defend the obtained results internally, yet, incorrect interpretations based on a one step dilution difference is still regarded as a deviation for the overall EQAS reporting, evaluation and in the database.

The EURL-AR is accredited by DANAK (accreditation no. 516) as provider of proficiency test for zoonotic pathogens and indicator organisms in bacterial isolates (serotyping, identification, and antimicrobial susceptibility testing).

## 2. Materials and Methods

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Detailed materials and methods are described in the network report (1).

From the EURL-AR-network, 30 countries delivered 35 sets of *Salmonella* results and 29 sets of *Campylobacter* results, and from the FWD-network, 9 countries delivered 8 sets of *Salmonella* results and 8 sets of *Campylobacter* results (App. 1). From seven countries, laboratories from both the public health and from the veterinary/food sector participated, from 23 and two countries, respectively, laboratories from the veterinary/food sector and the public health sector, only, participated.

Eight *Salmonella* strains and eight *Campylobacter* strains were selected for this trial among isolates from the strain collection at DTU Food. Individual sets of the *Salmonella* strains were provided as agar stab cultures and the *Campylobacter* strains as charcoal swabs. The process of preparation, assigning expected values, verification of expected values and shipment handling is described in detail in the EURL-AR network report (1).

The selection of antimicrobials used in the trial for *Salmonella* was: ampicillin, cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, ceftiofur, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, sulfonamides (sulfamethoxazole), tetracycline and trimethoprim. Additionally, cefoxitin was used for detection of ampC, and imipenem, imipenem/EDTA for detection of metallo-beta-lactamases.

For *Campylobacter* the following antimicrobials were included: chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin, and tetracycline.

In this EQAS, the protocol states that interpretative criteria which should be used were cut-off values recommended by the European Food Safety Authority (EFSA) and

listed in the protocol (1). The participants from the EURL-AR network were instructed to use the method carried out when performing monitoring for EFSA, whereas the participating public health laboratories could perform the AST using their method routinely employed in their laboratory. In general, participants using DD (*Salmonella* and *Campylobacter*) and E-test (*Campylobacter*) were recommended to interpret their results according to their individual routine, categorising the test strains into the terms resistant and susceptible. A categorisation as 'intermediate' was not accepted.

In general, agar and broth dilution methods are considered the gold standard as regards antimicrobial susceptibility testing, and the EURL-AR recommends using these methods when performing AST. For *Campylobacter*, the EURL-AR does not recommend the use of either disk diffusion or E-test for AST; i.e. the only type of method recommendable for AST of *Campylobacter* is dilution methods. According to the protocol, the laboratories of the FWD-network could submit results of AST of *Campylobacter* obtained by in-house methods like disk diffusion or E-test, in which cases in-house interpretative criteria should be applied as described in the protocol.

For the EURL-AR network, the detection of ESBL-producing strains was mandatory, whereas it was an optional part of the EQAS for the FWD-network laboratories.

The participants were instructed to enter results from the quality control (QC) reference strains into the database for use as background for the analysis of the obtained results (1). The evaluated results would consist of MIC values or inhibition zone diameters in millimetres for the reference strain *E. coli* (ATCC 25922) and MIC values for *C. jejuni* (ATCC 33560). The results should be in agreement with the quality

control ranges according to the relevant guidelines; i.e. the CLSI documents M31-A3 (2008) or M100-S22 (2012); The Sensititre System (Trek Diagnostic Systems Ltd, UK); or E-tests (AB-Biodisk, Sweden).

The database generated evaluation reports assessed the submitted results, describing all deviations from the expected. Deviations in the interpretation as resistant or susceptible were categorised as 'incorrect', as was also deviations in confirmation of an isolate as ESBL-producer or ampC.

There are two different types of interpretative criteria of results, clinical breakpoints and epidemiological cut-off values. The terms 'susceptible', 'intermediate' and 'resistant' should in principle be reserved for classifications made in relation to the therapeutic application of antimicrobial agents. When reporting data using epidemiological cut-off values, bacteria should be reported as 'wild-type' or 'non-wild-type' (2). Due to the different methods of AST used by the participants and also to simplify the interpretation of results, throughout this report, we will still maintain the terms susceptible and resistant, even in the cases where we are referring to wild-type and non-wild-type strains. The resistance profiles of the included test strains are available in the EURL-AR EQAS report (1).

The database evaluation was based on the submitted interpretation of each of the strain/antimicrobial combinations. After the conclusion of the EQAS, it appeared that some laboratories had obtained evaluations as incorrect due to an obtained MIC at the expected level which was interpreted according to other criteria than those in the protocol. This in particular was an issue for the testing of *Salmonella* isolates towards ciprofloxacin. The protocol states that if applying clinical breakpoints, the difference between the clinical breakpoint from Clinical and Laboratory Standards Institute (CLSI) and the

epidemiological cut off values recommended by EUCAST (European Committee on Antimicrobial Susceptibility Testing) could cause an obtained MIC-result to be incorrectly categorized and thereby deviate from the expected interpretation in this EQAS. In addition, for disk diffusion results, the protocol describes that the obtained result should be interpreted according to the laboratory's individual breakpoints, categorising them into the terms resistant and susceptible. It should be noted that the public health laboratories and also some of the laboratories in the EURL-AR network in general are performing AST for clinical purposes, and therefore as a routine are interpreting AST results according to clinical breakpoints.

When first analysing the overall EQAS results, it was evident that this difference in interpretative criteria for ciprofloxacin/*Salmonella* between the clinical breakpoints and the epidemiological cut off values, was the cause of many of the obtained deviations; in particular for results obtained by AST performed by disk diffusion.

Subsequently to the finalization of the EQAS, it was therefore decided that analysis and comparison of data for this report should be based on re-interpretation according to the criteria listed in the protocol of the obtained MIC-results that had caused deviations. For AST performed by disk diffusion of *Salmonella* towards ciprofloxacin, the criteria indicated by the data presented in the publication by Cavaco and Aarestrup, 2009 (3) were applied; that *Salmonella* isolates exhibiting an inhibition zone  $\leq 30$  when tested towards a 5µg ciprofloxacin disk should be regarded resistant to ciprofloxacin.

The database included questions for evaluation of the EQAS as well as questions regarding the individual laboratories' work in the area of AST. Few laboratories made use of this possibility of sending comments to the EURL-AR; those who did have received direct reply when relevant.



### 3. Results and discussion

The reported results included MIC values or inhibition zone diameters obtained by disk diffusion (DD) together with the categorisation as resistant or susceptible. Only the categorisation was evaluated, whereas the MIC values and disk diffusion inhibition zones were used as supplementary information.

The EURL-AR network has agreed that if less than 75% of the results were correct, based on strain/antimicrobial combination, these results should be further analysed and possibly omitted from evaluation. In the present EQAS this occurred in one case which was omitted from evaluation. This concerns the combination of the test strain C-7.1/tetracycline with a level of agreement with the expected results at 71%, when assessing the results obtained by the EURL-AR network. Consequently, all results from this strain/antimicrobial combinations have been omitted in this analysis.

The methods listed in Table 1 were used for AST by the laboratories of the FWD-network and the EURL-AR-network. No participants submitted AST results obtained by disk diffusion for *Campylobacter*.

The percentages of deviations from the expected results of AST performed by laboratories from each of the networks are illustrated in Figure 1. As indicated, both results obtained by each of the networks are below the 5% acceptance level.

Figures 2 and 3 illustrate the total percentage of deviations from the expected results obtained by the different methods performed, divided between each of the networks for AST's

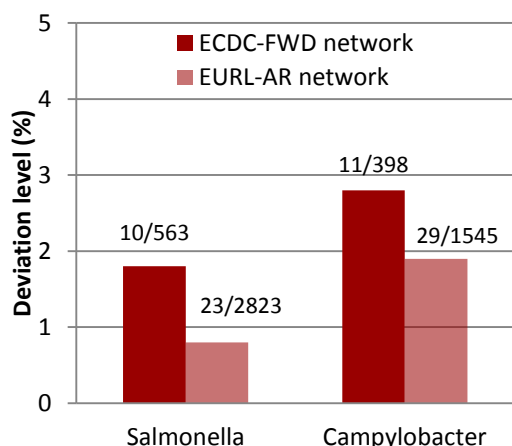
performed on *Salmonella* and *Campylobacter* test strains, respectively. For AST of *Salmonella*, a significant difference ( $\chi^2$ -test;  $p < 0.01$ ) was obtained when comparing results from both networks obtained by the use of disk diffusion and a MIC method with the MIC-determination exhibiting the better result. For the *Campylobacter* AST, the results presented for MIC determination are based on a lower number of tests for the FWD-network (N=110) compared to the EURL-AR network (N=1545) and shows a higher deviation level for the FWD-network (Figure 1). Laboratories from the FWD-network performed AST of *Campylobacter* by E-test, and a comparison of MIC results obtained by microbroth or agar dilution (both networks) shows no significant difference to those obtained by E-test (Fishers exact;  $p = 0.8$ ).

As for the recommendation by the EURL-AR that the only type of method recommendable for AST of *Campylobacter* is dilution methods, i.e. broth or agar dilution methods, this is based on the fact that internationally recommended interpretative criteria are available for broth and agar dilution methods, only. These methods have been validated and are recommended by CLSI ([www.clsi.org](http://www.clsi.org)) and EUCAST ([www.eucast.org](http://www.eucast.org)). It should be noted, however, that for disk diffusion, EUCAST has recently (February 2013) issued a standardised method based on Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/liter  $\beta$ -NAD (MH-F plate) and a higher incubation temperature ( $41 \pm 1^\circ\text{C}$ ), whereas for E-test there are for the moment no international references for quality assurance and interpretative criteria.

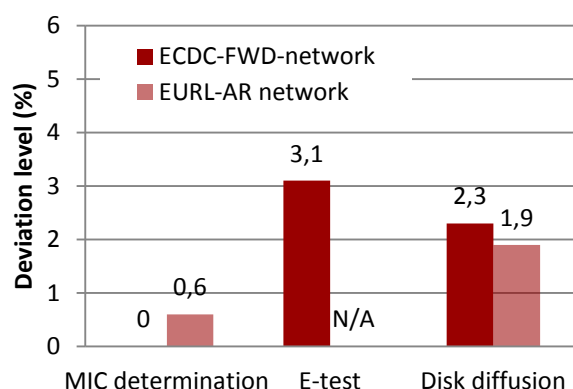
**Table 1:** Number of laboratories using each method for AST in this proficiency test

	<i>Salmonella</i>			<i>Campylobacter</i>		
	Microbroth or agar dilution	E-test	Disk diffusion	Microbroth or agar dilution	In-house E-test	In-house disk diffusion
ECDC FWD network	2	1	5	2	6	-
EURL-AR network	30	-	5	29	-	-

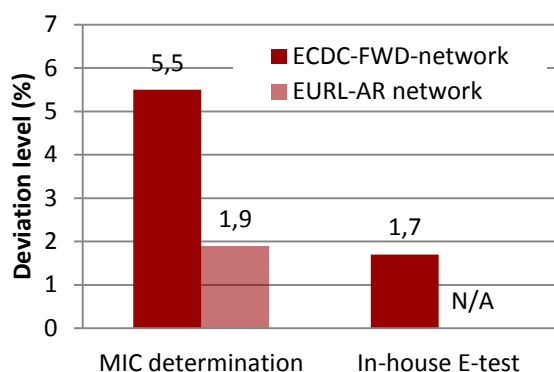




**Figure 1:** A comparison between the results obtained by the FWD-network and the EURL-AR network showing the total percentage of deviations for AST.



**Figure 2:** The percentage of deviations (number of deviations relative to the total tests performed) for AST's of *Salmonella* test strains performed using each of the available methods. N/A: Not applicable.



**Figure 3:** The percentage of deviations for AST's of *Campylobacter* test strains performed using each of the available methods. N/A: Not applicable.

Figures 4 and 5 illustrate the total percentage of deviations from the expected results obtained by each of the laboratories divided between each of the networks for AST's performed on *Salmonella* and *Campylobacter* test strains, respectively. The laboratories are ranked according to their performance determined by the percentage of deviating results in tests including all antimicrobials but excluding ESBL confirmatory tests.

Assessing results obtained by both networks, the deviation level for the *Salmonella* AST is generally low, with three laboratories exhibiting deviation levels at 6-7%, and with 40 laboratories (93%) performing acceptably according to the acceptance level at 5%.

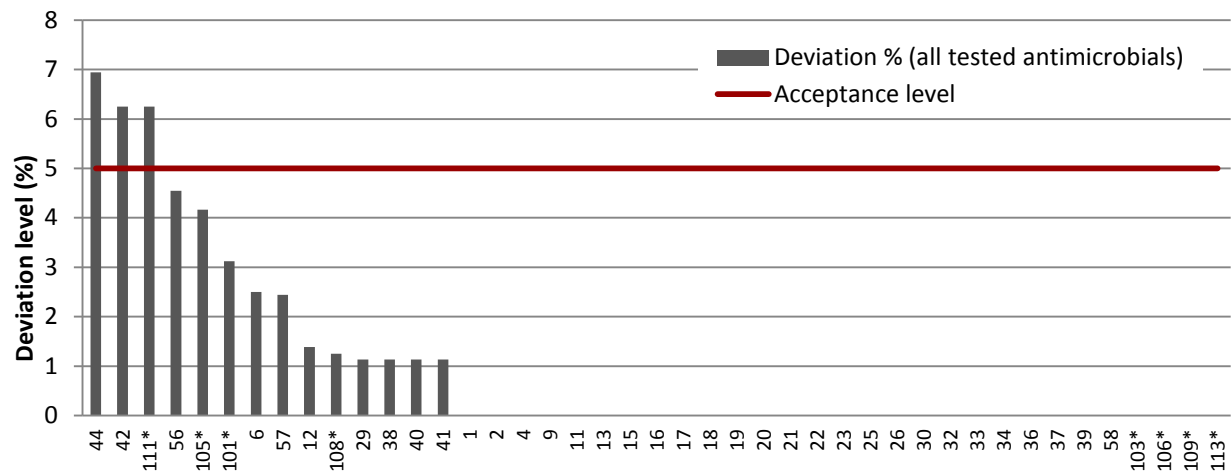
For the *Campylobacter* AST, 30 (81%) laboratories submitted results which meet the acceptance level (<5%). Of the seven laboratories with a higher deviation level, one laboratory (#44) exhibited a level of 14.5% deviations and has informed the EQAS organizers that the method has been reviewed and radical changes introduced.

Figures 6 and 7 illustrate the total percentage of deviations from the expected results on each of the antimicrobials divided between each of the networks for AST's performed on *Salmonella* and *Campylobacter* test strains, respectively.

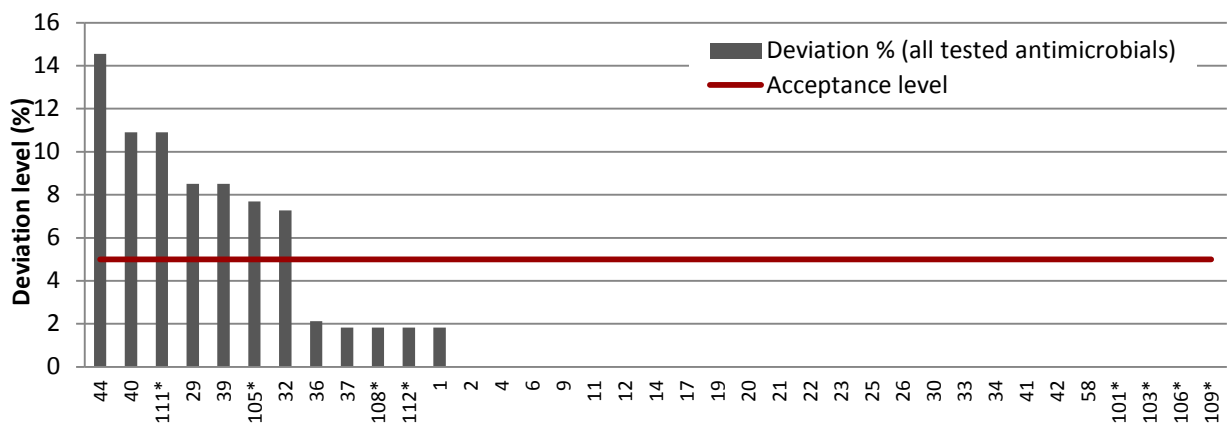
For *Salmonella*, the results for sulfamethoxazole shows the highest deviation level for the FWD-network laboratories (8.3%), and is also the antimicrobial that lead to the highest deviation level for the EURL-AR-network (2.1%). Almost all deviations for this antimicrobial were caused by a false resistant result which is likely to be due to the reading of the result. It should be noted that when reading the AST-result obtained for *Salmonella*/sulfamethoxazole, the MIC-value or disk diffusion zone should be determined at 80% inhibition of growth due to the fact that this drug is bacteriostatic and not bactericidal.

For ciprofloxacin, the interpretative criteria listed in the protocol refer to EUCAST where the cut off value for this antimicrobial is 0.06 µg/mL which for the time being has no corresponding zone diameter available for interpretation of disk diffusion results and is considerably lower than the clinical breakpoint set by CLSI (4 µg/mL). Four of the *Salmonella* strains exhibited low-level resistance to ciprofloxacin, and one of these harboured a plasmid-mediated quinolone resistance (PMQR) gene (*S-7.8/qnrS1*) and thus exhibits nalidixic acid susceptibility and low-level ciprofloxacin resistance, the latter not being detectable when applying the routine

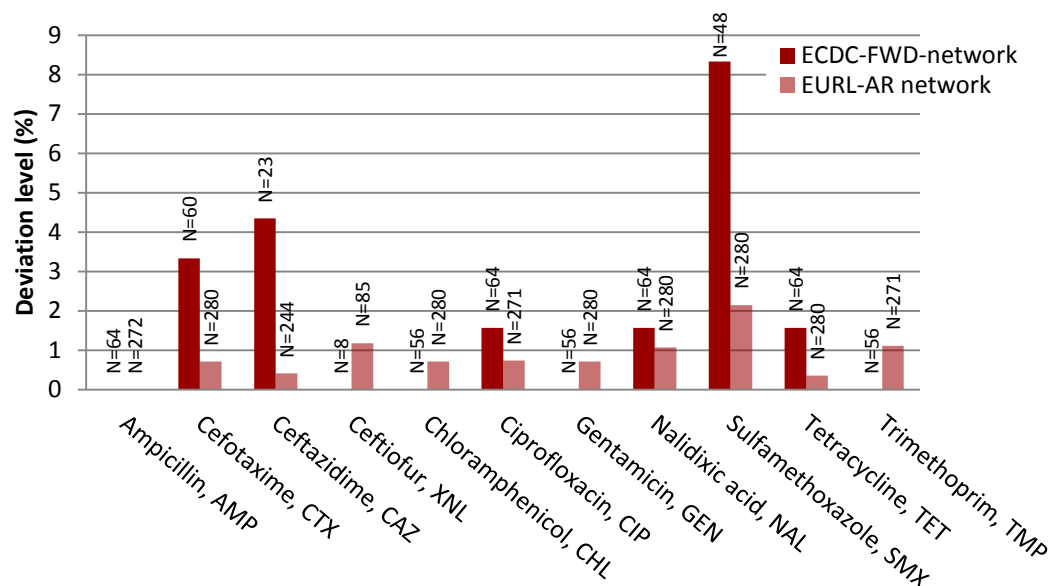
CLSI methods and guidelines. The consequence of obtaining an incorrect interpretation in this EQAS, i.e. when applying the epidemiological cut off values for interpretation, is however not necessarily an incorrect interpretation in a clinical context. When analysing according to epidemiological cut off values, it is recommended that laboratories performing disk diffusion for AST of *Salmonella* refer to the publication by Cavaco and Aarestrup, 2009 (3), which describes suggestions for disk content and indicates cut off values for AST by disk diffusion of *Salmonella* isolates harbouring a PMQR-gene.



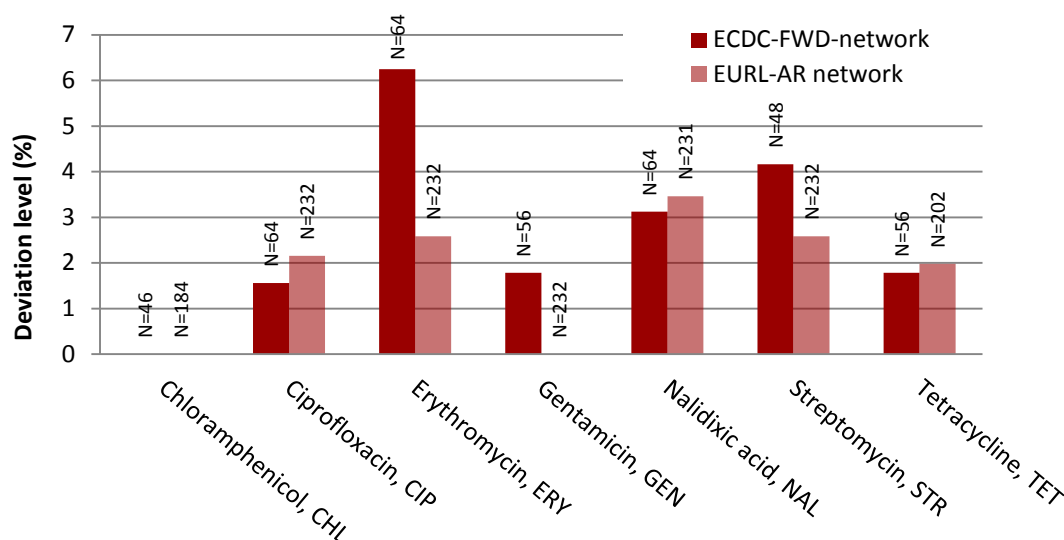
**Figure 4:** Individual participants' deviations in percent of their total number of *Salmonella* AST's. Laboratory numbers below 100 belong to the EURL-AR-network, whereas laboratory numbers from 101-111 are indicated with an asterisk and belong to the FWD-network



**Figure 5:** Individual participants' deviations in percent of their total number of *Campylobacter* AST's. Laboratory numbers below 100 belong to the EURL-AR-network, whereas laboratory numbers from 101-111 are indicated with an asterisk and belong to the FWD-network.

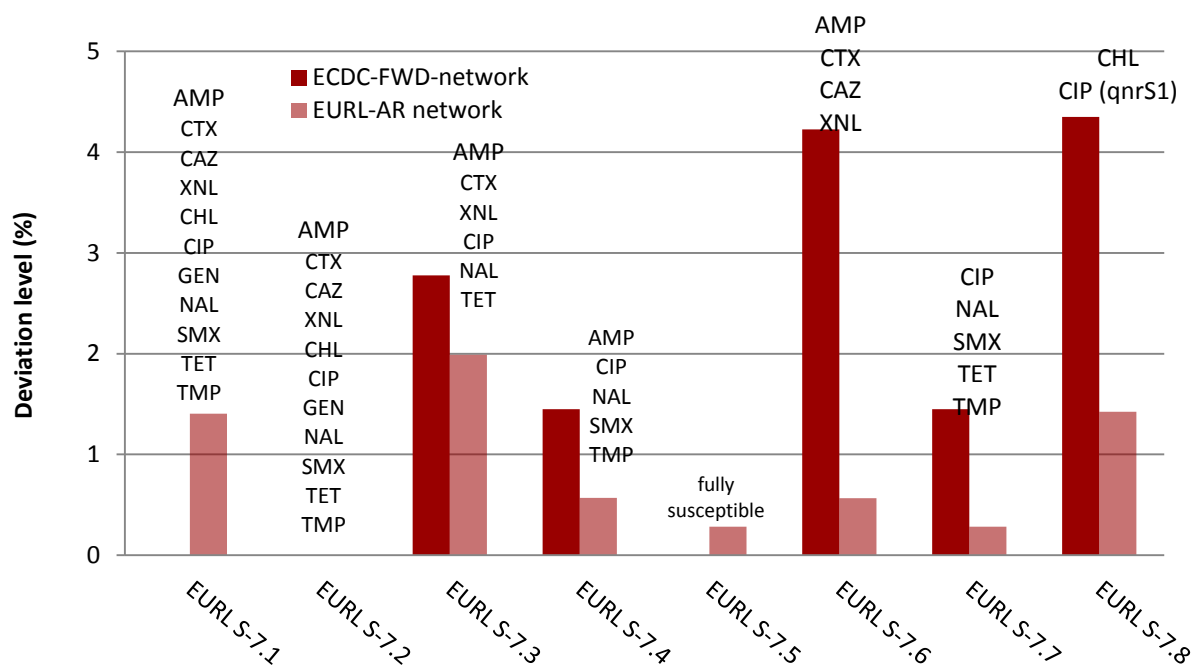


**Figure 6:** The percentage of deviations on each of the antimicrobials for AST's performed on *Salmonella* test strains. Above each bar, the numerator and denominator are given<sup>1</sup>.

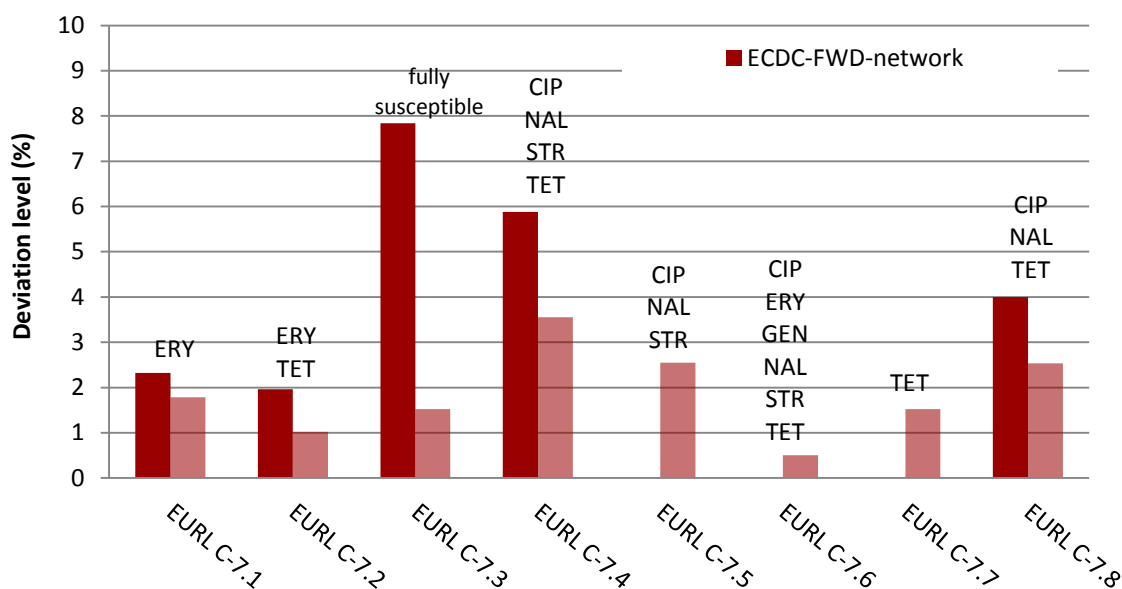


**Figure 7:** The percentage of deviations on each of the antimicrobials for AST's performed on *Campylobacter* test strains. Above each bar, the numerator and denominator are given.

<sup>1</sup> Prior to the re-interpretation described in the 'Materials and methods'-section, the deviation level for ciprofloxacin was high. Many of the former deviations could be attributed to the interpretative criteria applied in this EQAS which refer to EUCAST. For ciprofloxacin, the epidemiological cut off value is 0.06 µg/mL which is very low compared to the CLSI clinical breakpoint (R≥4 µg/mL). Also, EUCAST does not provide a zone diameter corresponding to the low MIC cut off value. Therefore laboratories applying the routine CLSI methods and guidelines for the testing of the four *Salmonella* strains exhibiting low-level resistance to ciprofloxacin have obtained deviating results from the expected.



**Figure 8:** The percentage of deviations on each of the test strains for AST's performed on *Salmonella* test strains. When a strain exhibited resistance to a certain antimicrobial it is indicated by an antimicrobial code; i.e. AMP, ampicillin; CTX, cefotaxime; FOX, ceftiofur; CAZ, ceftazidime; XNL, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; SMX, sulphonamides; TET, tetracycline; and TMP, trimethoprim<sup>2</sup>.



**Figure 9:** The percentage of deviations on each of the test strains for AST's performed on *Campylobacter* test strains. For each of the strains, a resistance phenotype is indicated by an antimicrobial code; i.e. CHL, chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; and TET, tetracycline.

<sup>2</sup> See footnote 1

For the *Salmonella* AST, the FWD-network and the EURL-AR-network, respectively, on average tested 8.8 and 10.1 antimicrobials per test strain. A number of the laboratories from the FWD-network did not test ceftiofur (veterinary antimicrobial) and ceftazidime. The same was the case for the ceftiofur for the EURL-AR-network.

For *Campylobacter*, the FWD-network laboratories demonstrated 11 (2.8%) deviations, all of which were interpretations as resistant where the expected result was susceptible. One laboratory counted for six of these deviations. As for the EURL-AR-network, the deviations (n=29; 1.9%) were caused by various reasons encompassing inappropriate laboratory procedures, transcription errors and application of interpretative criteria other than those in the protocol.

In total for *Campylobacter*, the FWD-network and the EURL-AR-network on average tested 6.2 and 6.7 antimicrobials per test strain, respectively.

Figures 8 and 9 illustrate the total percentage of deviations from the expected results obtained for each of EQAS test strains divided between each of the networks for AST's performed on *Salmonella* and *Campylobacter* test strains, respectively. The resistance phenotype of each of the strains is indicated.

For *Salmonella* (Figure 8), three strains caused very few deviations or none (S-7.1, S-7.2, and S-7.5) whereas the remaining five strains accounted for a number of deviations for both the networks. All strains that did not exhibit resistance to sulphonamides were to some extent incorrectly categorized as resistant to this antimicrobial by five laboratories; two from the FWD-network and three from the EURL-AR network.

For *Campylobacter*, the deviations from the FWD-network laboratories belong to five strains (C-7.1, C-7.2, C-7.3, C-7.4, and C-7.8), whereas

for the EURL-AR-network, the deviations are spread over all eight test strains with one laboratory contributing to the deviations for six of the eight test strains.

#### ESBL-producing *Salmonella* test strains

For the EURL-AR network, the detection of ESBL-producing strains was mandatory, whereas it was an optional part of the EQAS for the ECDC FWD-network laboratories. The details of the ESBL-detection and confirmation are addressed in the EURL-AR report (1).

As indicated in Table 2, four test strains; S-7.1, S-7.2, S-7.3 and S-7.6 were ESBL-producers, i.e. three were so-called 'true ESBLs' whereas one was an *ampC*-producing strain.

The majority of the 35 participants (n=30, 32, and 31) from the EURL-AR network confirmed the ESBL-production for the strains S-7.1, S-7.2, and S-7.3, whereas 11 (31%) confirmed the *ampC*-positive strain, S-7.6. Of the eight FWD-network laboratories submitting results on the *Salmonella* test strains, seven participated in the ESBL component and all submitted results for the strains S-7.1, S-7.2, and S-7.3 were in agreement with the expected. For the *ampC*-positive strain (S-7.6), however, only three of the laboratories confirmed *ampC*-production. Of note, the strain S-7.6 had an unusual phenotype/genotype. The strain exhibited resistance to

**Table 2:** ESBL-producing *Salmonella* test strains.

Test strain	Genes conferring resistance to beta-lactam antimicrobials	True ESBL or <i>ampC</i> -producer
S-7.1	<i>bla</i> <sub>CTX-M-15</sub>	True ESBL
	<i>bla</i> <sub>OXA-30</sub>	
	<i>bla</i> <sub>TEM-1</sub>	
S-7.2	<i>bla</i> <sub>CTX-M-15</sub>	True ESBL
	<i>bla</i> <sub>OXA-10</sub>	
	<i>bla</i> <sub>TEM-1</sub>	
S-7.3	<i>bla</i> <sub>CTX-M-9</sub>	True ESBL
	<i>bla</i> <sub>TEM-1</sub>	
S-7.6	<i>bla</i> <sub>ACC-1</sub>	<i>ampC</i> -producer

cefotaxime and ceftazidime (and ceftiofur), but when trying to confirm the ESBL-production by testing for synergy with clavulanic acid, ESBL-production could not be confirmed, nor did the strain show resistance to ceftiofur. Consequently, the results from the phenotypic testing could not confirm ampC- or ESBL-production. In a genotypic analysis, however, the strain was found to harbour *bla*<sub>ACC-1</sub>. The organizers concluded that the fact that this strain is phenotypically resistant to cephalosporins should induce the participant to suspect that the strain harboured one type or another of ESBL- or ampC-producing gene and should then demand further investigation, including molecular testing

#### Deviations by reference strains

In the following section, deviations are defined as results of antimicrobial susceptibility tests on the reference strain that are outside the quality control (QC) acceptance intervals (App. 2). All but one of the laboratories from the FWD-

network submitted results for the quality control of the *Salmonella* AST (7 laboratories), and for the *Campylobacter* AST, all uploaded QC-data (8 laboratories). For the EURL-AR-network, all 29 laboratories performing MIC for AST of *Campylobacter* uploaded QC-results, and also all but one of the 35 laboratories submitting results for *Salmonella*.

The results from the reference strain should be assessed at the laboratory as part of the quality assurance of the values obtained when performing AST on the test strains, and are therefore especially important for laboratories which have deviations listed in their evaluation report.

The submitted results from testing the *C. jejuni* reference strain could be evaluated in the EQAS-database for two of the eight FWD-network laboratories, as the remaining uploaded values were E-test MIC-values where no evaluation criteria is available.

## 4. Conclusions

The objective of providing the EURL-AR EQAS to the FWD-network was to assess and compare the quality of the antimicrobial susceptibility data produced by the reference laboratories from the two networks. In addition, it was to identify areas which would require attention to produce reliable and harmonised susceptibility data.

The number of participating laboratories varied between the two networks, with eight laboratories from the FWD-network for both the *Salmonella* and the *Campylobacter* component, and 35 and 29 participating laboratories, respectively, from the NRL-AR network.

This assessment demonstrates that the AST-results obtained by the FWD-network and the EURL-AR-network for *Salmonella* and *Campylobacter* are comparable when applying

the acceptance level of 5% for deviations for each laboratory. This goal was met for 7 (87.5%) of the FWD-network laboratories and for 33 (94%) of the NRL-AR's for *Salmonella* AST. For *Campylobacter* AST this was the case for 6 (75%) of the FWD-network laboratories and for 24 (83%) of the NRL-AR's.

At the time when this EQAS was conducted (October-December 2012), dilution methods, only (i.e. broth or agar dilution methods), were recommended by the EURL-AR for AST of *Campylobacter*. Subsequently (February 2013), EUCAST issued a standardised method for AST of *Campylobacter* by disk diffusion. For the moment, no international references for quality assurance and interpretative criteria are available for E-test of *Campylobacter*. In this EQAS, however, the deviation level for results obtained by E-test was at 1.7% and disk

diffusion was not applied by any of the participants for AST of *Campylobacter*.

Especially for the FWD-network laboratories, the interpretation of ciprofloxacin posed a problem. Many laboratories in this network perform DD for AST of *Salmonella* and as this breakpoint is much lower than the clinical breakpoint, this generates a difference in interpretation.

The issue about detection of ESBL-producing *Enterobacteriaceae* is critically relevant for both the public health laboratories and the laboratories from the veterinary/food sector as these phenotypes appear to continue to emerge worldwide. Laboratories which have not yet introduced tests to detect ESBL-producing *Enterobacteriaceae* are therefore encouraged to prioritize this area.

## 5. References

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## Participant list

<i>Salmonella</i>	<i>Campylobacter</i>	Sector	Institute	Country
X	X	Veterinary/Food	Austrian Agency for Health and Food Safety	Austria
X	X	Veterinary/Food	Institute of Public Health	Belgium
-	X	Public Health	Saint-Pierre university Hospital & Jules Bordet Institute	Belgium
X	X	Veterinary/Food	Nacional Diagnostic and Research Veterinary Institute	Bulgaria
X	-	Veterinary/Food	Croatian Veterinary Institute	Croatia
X	X	Veterinary/Food	Veterinary Services	Cyprus
X	X	Veterinary/Food	State Veterinary Institute Praha	Czech Republic
X	X	Veterinary/Food	National Food Institute	Denmark
X	X	Public Health	Statens Serum Institut	Denmark
X	X	Veterinary/Food	Estonian Veterinary and Food Laboratory	Estonia
X	X	Veterinary/Food	Finnish Food Safety Authority EVIRA	Finland
X	X	Public Health	National Institute for Health and Welfare (THL)	Finland
X	-	Veterinary/Food	ANSES Fougères	France
X	X	Veterinary/Food	ANSES Lyon	France
X	-	Veterinary/Food	ANSES Maisons Alfort	France
-	X	Veterinary/Food	ANSES Ploufragan	France
X	X	Veterinary/Food	Federal Institute for Risk Assessment	Germany
X	-	Veterinary/Food	Veterinary Laboratory of Chalkis	Greece
X	X	Veterinary/Food	Central Agricultural Office, Veterinary Diagnostical Directorate	Hungary
X	X	Public Health	Landspítali University Hospital	Iceland
X	X	Veterinary/Food	Central Veterinary Research Laboratory	Ireland
X	X	Veterinary/Food	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
X	X	Veterinary/Food	Institute of Food Safety, Animal Health and Environment „BIOR”	Latvia
X	X	Veterinary/Food	National Food and Veterinary Risk Assessment Institute	Lithuania
X	X	Public Health	Laboratoire National de Santé	Luxembourg
X	X	Veterinary/Food	Public Health Laboratory	Malta
X	X	Veterinary/Food	Central Veterinary Institute of Wageningen UR	Netherlands
X	X	Public Health	Norwegian Institute of Public Health	Norway
X	X	Veterinary/Food	Veterinærinstituttet	Norway
X	X	Veterinary/Food	National Veterinary Research Institute	Poland
X	X	Veterinary/Food	Laboratório Nacional de Investigação Veterinária	Portugal
X	X	Veterinary/Food	Institute for Diagnosis and Animal Health	Romania
X	X	Veterinary/Food	Institute for Hygiene and Veterinary Public Health	Romania
X	X	Veterinary/Food	State Veterinary and Food Institute (SVFI)	Slovakia
X	X	Public Health	Institute of Public Health of the Republic of Slovenia	Slovenia
X	X	Veterinary/Food	National Veterinary Institute	Slovenia
X	-	Veterinary/Food	Centro nacional de Alimentación. Agencia Española de Seguridad	Spain
X	X	Public Health	Instituto de Salud Carlos III (ISCIII)	Spain
X	X	Veterinary/Food	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	X	Veterinary/Food	National Veterinary Institute, SVA	Sweden
X	X	Veterinary/Food	Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland
X	-	Veterinary/Food	National Food Reference Laboratory	Turkey
X	-	Public Health	Scottish Salmonella, Shigella & C. difficile Reference Laboratory	United Kingdom
X	X	Veterinary/Food	Centre for Infections Health Protection Agency	United Kingdom
X	X	Veterinary/Food	The Veterinary Laboratory Agency	United Kingdom

	Designated NRL-AR by the competent authority of the member state
	Not a Member State of the EU

## QC ranges for reference strains

<i>E. coli</i> ATCC 25922		
Antimicrobial	MIC	DD (disc content)
Ampicillin, AMP	2-8	16-22 (10µg)
Cefotaxime, CTX	0.03-0.12	29-35 (30µg)
Cefoxitin, FOX	2-8	23-29 (30µg)
Ceftazidime, CAZ	0.06-0.5	25-32 (30µg)
Ceftiofur, XNL	0.25-1	26-31 (30µg)
Chloramphenicol, CHL	2-8	21-27 (30µg)
Ciprofloxacin, CIP	0.004-0.016	30-40 (5µg)
Gentamicin, GEN	0.25-1	19-26 (10µg)
Imipenem, IMI	0.06-0.25	26-32 (10µg)
Nalidixic acid, NAL	1-4	22-28 (30µg)
Sulfisoxazole, FIS	8-32	15-23 (250/300µg)
Tetracycline, TET	0.5-2	18-25 (30µg)
Trimethoprim, TMP	0.5-2	21-28 (5µg)

MIC ranges and disc diffusion ranges are according to CLSI M100 S22 with the following exceptions:

E-test ranges are according to AB-Biodisk

<i>Campylobacter jejuni</i> ATCC 33560				
Antimicrobial	Microbroth (36-37°C/48h)	Microbroth (42°C/24h)	Agar dilution (36-37°C/48h)	Agar dilution (42°C/24h)
Chloramphenicol, CHL	1-8	1-4	None	None
Ciprofloxacin, CIP	0.06-0.25	0.03-0.12	0.12-1	0.06-0.5
Erythromycin, ERY	0.5-2	0.25-2	1-8	1-4
Gentamicin, GEN	0.5-2	0.25-2	0.5-2	0.5-4
Nalidixic acid, NAL	4-16	4-16	None	None
Tetracycline, TET	0.25-2	0.25-1	None	None

Ranges are according to CLSI (M31-A3)

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